

Tibia organ culture and Col10a1 RNAScope

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 An abbreviated version of this protocol was published in Science Translational Medicine in May 2021

An RNA aptamer restores defective bone growth in FGFR3-related skeletal dysplasia in mice

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Detailed protocol

Tibias from E18 mouse embryos (ICR line, Masaryk University) were dissected and placed in PSA (0.4g KCl, 8g NaCl, 0.35g NaHCO₃, 1g glucose in 1000ml H₂O, pH7.2, sterile filtered). Soft tissues were gently removed without disturbing epiphyses. Tibias were placed on Millipore filters above a metal mesh and cultured at air-liquid interface in F12/DMEM supplemented with 10% FBS, ascorbic acid (50 ug/ml), and 10 mM beta-glycerol phosphate for 8 days. Media supplemented with FGF2 (100 ng/ml), RBM-007 (100 nM), or scrambled aptamer (100 nM) were changed daily. Tibia were photographed and length of the tibias was measured from pictures by day 0 and at the end of cultivation (day 8) in Axio Vision (Zeiss). For histology, tibias were fixed in 4% paraformaldehyde (PFA for 24h), decalcified in 10% EDTA (pH7.2, 24h, two changes of the solution), washed thoroughly in DEPC H₂O, dehydrated in ethanol series (70% EtOH for 1h, 80% EtOH for 1h, 90% EtOH for 1h, 100% EtOH for 1h), cleared 3x in xylene for 1h, paraffin infiltration 2x1h + ON, embedded in paraffin. Paraffin embedded samples were sectioned into 5um slices and placed onto microscopic slides and stained with hematoxylin and eosin. Col10a1 expression was detected on alternative sections by Mm-Col10a1 probe using RNAScope Technology (ACD Bio). The hybridized probe was visualized using the TSA-Plus Cyanine 3 system (Perkin-Elmer). DAPI was used to stain nuclei.

RNAScope is patented technology (acdbio.com). RNAScope Fluorescent Kit was used to detect Col10a1 and protocol was followed with no modifications (see attachment).

Related files

 320293_RNAScope_Multiplex_UM_11052013_a.pdf



How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Nakamura, Y., Ozono, K. and Krejci, P. (2021). Tibia organ culture and Col10a1 RNAScope. Bio-protocol Preprint. bio-protocol.org/prep1375.
2. Kimura, T., Bosakova, M., Nonaka, Y., Hrubá, E., Yasuda, K., Futakawa, S., Kubota, T., Faflek, B., Gregor, T., Abraham, S. P., Gomolkova, R., Belaskova, S., Pesl, M., Csukasi, F., Duran, I., Fujiwara, M., Kavkova, M., Zikmund, T., Kaiser, J., Buchtova, M., Krakow, D., Nakamura, Y., Ozono, K. and Krejci, P. (2021). An RNA aptamer restores defective bone growth in FGFR3-related skeletal dysplasia in mice. Science Translational Medicine 13(592). DOI: [10.1126/scitranslmed.aba4226](https://doi.org/10.1126/scitranslmed.aba4226)

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